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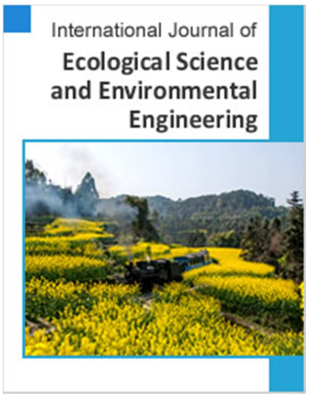

Heavy Metal Concentrations of *Citrus* Species (*Citrus reticulata* and *Citrus sinensis*) Cultivated on Road Sides in Uyo Metropolis in Akwa Ibom State, Nigeria

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Keywords

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Abstract

A field study was undertaken to establish heavy metals' (Cd, Cu, Mn, Pb and Zn) concentrations in soil and *Citrus* species samples from two locations in Uyo metropolis in Akwa Ibom State, Nigeria; L1 (Ebuk's farm, Atan Offot village), L2 (Ukpabio's farm, Ikot Akpan Abia village, Ibesikpo L. G. A.) using atomic absorption spectrophotometer. The results showed that the heavy metals (Cd, Cu, Pb, Mn and Zn) present in the soils were within World Health Organization (WHO) permissible limits. Similarly, the heavy metals concentrations of fruits were within WHO permissible limits. The highest concentration of Mn (7.818±0.624mg/kg) was in L2, Pb (0.676±0.164 mg/kg) and Zn (2.196±0.436mg/kg) were in L1. The levels of Cd, Cu, Mn, Pb and Zn in soil L1 were 0.212±0.036, 1.896±0.128, 0.676±0.164, 5.556±1.316 and 2.196±0.436mg/kg, L2 were 0.157±0.014, 1.157±0.285, 0.568±0.088, 7.818±0.624 and 1.403±0.311 respectively. In *Citrus* plants, the highest concentration of Pb was from *Citrus sinensis* (1.75±0.04 mg/kg) and Zn was from *Citrus reticulata* (6.27±0.10 mg/kg). The linear regression (graphs) result showed Cu and Mn of *Citrus reticulata*; Cd and Cu of *Citrus sinensis* which indicated that these plants have the ability to bioaccumulate these metals. The concentrations of metals in soil and *Citrus* plant were in decreasing order: Mn> Zn>Pb>Cd>Cu. The concentration of the heavy metals in the *Citrus* fruit and soil samples in this study could serve as a baseline data for the assessment of agricultural and vehicular exhaust pollutants in soils and *Citrus* fruit obtained in Akwa Ibom State of Nigeria. There should be regular monitoring of toxic heavy metal levels in soil, sewage and foods, to prevent their excessive build-up in the food chain.

1. Introduction

Heavy metal (HM) contamination in soil is a major concern because of their toxicity andthreat to human life and the environment [1]. Heavy metals are major components of petroleum hydrocarbon [2]. Emission from heavy traffic on roads contain lead (Pb), cadmium (Cd), zinc (Zn) and nickel (Ni), which are present in fuel as anti-knock agents [3], [4]. The deposition of vehicle derived metal and the relocation of metals deposited on road surface by air and runoff water have led to contamination of soil [5], [6]. Heavy metal uptake via the roots from contaminated soils and direct deposition of contaminants from the atmosphere onto plant surfaces can lead to plant contamination by HM [7]. Soil to plant transfer is one of the key processes of human exposure to HM through the food chain. Toxic heavy metals entering the ecosystem may lead to geo-accumulation, bio-accumulation and bio-magnifications. Heavy metals get into plants via adsorption which refers to binding of materials onto the surface or absorption which implies penetration of metals into the inner matrix. Both mechanisms can also occur [8]. They get accumulated in time in soils and plants and would have a negative influence on physiological activities of plants (e.g. photosynthesis, gaseous exchange and nutrient absorption) determining the reductions in plant growth, dry matter accumulation and yield [9].

The consumption of plants produced in contaminated areas, in addition to ingestion or inhalation of contaminated particles [10] from vehicular emissions, are two principal factors contributing to human exposure to metals [6]. Thus, the consumption of metal contaminated plants may lead to hazard of enriching human alimentary canal with toxic metals. There are three primary areas affected by heavy metals in humans. These are the nervous system, the cardiovascular system and blood cells. Metals such as mercury and lead can disrupt nerve cells. Lead, cadmium, nickel and mercury can affect blood cells. The build-up of heavy metals can cause damage to the liver, kidneys and the circulatory system. Lead can substitute for calcium, particularly in bone. In children, when bones are developing and inadequate quantity of calcium is taken, lead can accumulate in the bones. This can cause damage to nerve cells and the brain [11].

The determination of metal in environmental samples such as soils and plants is very necessary for monitoring environmental pollution [12]. Uyo as a growing city in south southern Nigeria has experienced a rapid urbanization and vehicular density in the last decades and the rapid increase in the number of vehicles exerts heavy pressure on the urban environment. Monitoring of the contamination of soil with heavy metals is of interest due to their influence on ground water and surface water and also on plants, animals and humans [9]. This work, therefore, is aimed at investigating (a) the levels of heavy metal contents in two different types of Citrus species (b) the levels of heavy metal contents of soils from the selected sites which the *Citrus* speciesare grown (c) assess the relationships between the soil and Citrus species heavy metal contents determined using linear regression graphs; and give a set of recommendations which should serve as means of solving problems identified in the research or as stepping stone to other researches.

2. Materials and Methods

2.1. Description of the Study Area

The study of heavy metal (HM) contents in soil and plants was carried out in Uyo metropolis in Akwa Ibom State. Two study sites were selected randomly for this study, namely; Ebuk's farm, Atan Offot village, Uyo L.G.A. (L1) and Ukpabio's farm, Ikot Akpan Abia village in Ibesikpo L.G.A. (L2), all in Akwa Ibom State. Akwa Ibom is a tropical rain forest areas in South-South Nigeria lying between latitudes 4°32' and 5°33'N, and longitudes 7°25' and 8°25'E; it has an average temperature range of 25.1 to 27.8°C and an annual rainfall range of 33-37.8mm with theland mass of 115km^2 and the population of 1,400 million persons/km^2 [13]. The area is marked with a distinct rainy season occurring between April and October and a dry season occurring between November and early March. The average relative humidity is about 80%, and up to 95% occurring at the peak of the rainy season [14].

2.2. Sample Collection and Analysis

Samples were collected from two (2) locations within Uyo metropolis between December 2012 and March 2013. In each location, 10 soil samples and 10 plant samples were collected. A soil auger was used to obtain two soil samples at the base of each plant to a rooting depth of 20 cm [15]. Two fruits were plucked from each species, preserved in bags and labeled accordingly. The soil and fruit samples were put in plastic bags and transferred to Aluminium Smelter Company of Nigeria (ASCON), Ikot Abasi in Akwa Ibom State for further treatment and analysis.

2.3. Laboratory Procedures

In the laboratory, the soil samples were air-dried at room temperature and ground in a wooden mortar to pass through a 2 mm mesh sieve and stored in labeled soil bags. Sub-samples were taken from each soil sample and analyzed for heavy metal contents (Cd, Cu, Pb, Mn and Zn). Samples were wet digested with a concentrated acid mixture (HNO_3 and $HClO_4$). Plant samples were digested with HNO_3 and $HClO_4$ in 5:1 ratio until a transparent solution was obtained. The soil and plant digested solutions were cooled to room temperature, filtered, transferred quantitatively to 50 and 25 ml volumetric flasks, respectively, made up to volume with distilled water and kept in clean plastic vials before metal analysis [7]. Triplicate digestion of each sample together with a blank was also carried out. Thereafter, quantificationof metallic content of digested samples was carried out with a flame atomic absorption spectroscopy AAS (UNICAM 919 model).

2.4. Statistical Analysis

Statistical package for Social Sciences (SPSS, Version 20.0) was employed for Regression Analysis (SMRA) in order to determine the relationship between soil and species Heavy metals contents while Graph Pad Prism 5 was employed for Two Way Analysis of Variance (ANOVA) to identify if there was any significant difference among the data collected [16].

3. Results and Discussion

HM Concentration in soil/ Citrus Fruits: Table 1 and 2 represent the means (± S.E) of heavy metal contents in the soil and Citrus fruits of the study areas. Analysis of variance (ANOVA) indicates that there was no significant difference at P = 0.05 among values obtained for soil and citrus fruits, heavy metals in different locations and species, respectively. The results also showed that the heavy metals (Cd, Cu, Pb, Mn and Zn) present in the soils were within World Health Organization (WHO) permissible limits. Similarly, the heavy metals concentrations of fruits were within WHO permissible limits.

Table 1. *Mean (+ S.E.) of Heavy Metal Contents of two selected soil sites in Uyo Metropolis of Akwa Ibom State.*

		Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
L1	mg/kg	0.212±0.036	1.896±0.128	0.676±0.164	5.556±1.316	2.196±0.436
L2	mg/kg	0.157±0.014	1.157±0.285	0.568±0.088	7.818±0.624	1.403±0.311
Safelimit*[32]		3-6	135-270	250-500	-	300-600

Table 2. *Mean (±S.E) of heavy metal contents of Citrus reticulata and Citrus sinensis from selected locations in Akwa Ibom State.*

	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Citrus reticulata	0.45±0.04	0.55±0.08	1.34±0.09	17.31±2.38	6.27±0.10
Citrus sinensis	0.84±0.14	1.14±0.08	1.75±0.04	13.51±2.74	4.41±0.34
Safe limit* [32]	1.5	30	2.5	-	50

From the study, elevated Cd was observed in the Citrus fruits. The source of Cd in this study may be attributed to vehicular emissions. Cadmium is released as a combustion product in the accumulators of motor vehicles or in carburetors [17], lubricants [18], wearing of paints on the body of vehicles [6], tear and wear of tyres [19]. The ultimate sink for HM is atmospheric deposition and burial in soil [20]. They (HM) often accumulate in the top layer of soil, therefore, are available for uptake by plants via roots, which is a major entry point of HM that ultimately affects different physiological processes. Cadmium has no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic [21], [22] and long term exposure results in fragile bones and decreased bone strength [23], renal dysfunction, characterized by tubular proteinuria [24] in humans, severe irritation of the stomach leading to vomiting and diarrhea which can sometime result in death, kidney damage [23]. Cadmium is also a proven human carcinogen [23]. Thus, households or individuals that have been making uses of *Citrus reticulata* and *Citrus sinensis* from the study site may have predisposed themselves to serious health risk. The range of Cd in this study is 0.45±0.04 to 0.84±0.14mg/kg which is higher than 0.02±0.01 mg/kg in the leaves of *Psidiumgua java* in Aba (Abia State)[6] and 0.08±0.01 - 0.21±0.01 mg/g in leaves of *Saba florida* in Nigeria [25]. Indeed, metal contamination in urban soils of increasing concern due to food safety issues and potential health risks associated with intake of contaminated plant products [26].

The highest concentration of Pb in soil and fruits samples were in L1 (0.676±0.164 mg/kg) and *Citrus sinensis* (1.75±0.04 mg/kg), respectively. Lead (Pb) concentration in the samples may be attributed to vehicular emissions since there was no other visible source of pollution in the study site. Lead pollution in urban soil comes from combustion of gasoline that contains tetraethyl lead as anti-knock agent [6]. Zinc and Pb are amongst the metal referred to as common urban pollutants [27], [28]. Lead (Pb) was determined in topsoils in South Australia, up to 50 km from any major road and this was attributed to petrol exhaust, as no other sources could be reasonably identified in the region [29]. In addition, Pb released from exhaust emissions [30] into the atmosphere may be deposited on leaf surfaces and absorbed inside plant cells [6] where it may affect a number of cytoplasmic enzymes [31]. Though the concentration of Pb in this study is within the World Health Organization [32] safety limit, care must be taken on the consumption of *Citrus sinensis* and *Citrus reticulata* from the study area as these plants have elevated levels of Lead and the capacity to bioaccumulate it. Hence regular check is necessary to avoid a possible build up in the plants in future. Its (Pb) poisoning causes inhibition of synthesis of haemoglobin; cardiovascular system and acute and chronic damage to the central nervous system and peripheral nervous system [33], poor development of grey matter in the brain of children, resulting in poor intelligent quotient [34]. The range of Pb in this study is 1.34±0.09 to 1.75±0.04mg/kg, which is higher than 0.17± 0.02 mg/kg in leaves of *Saba florida* in Nigeria [25] but lower than 10.47±0.93 mg/kg in leaves of *Psidiumgua java* in Aba [6].

The highest concentrations of Zn in soil and fruit samples were in L1 (2.196±0.436 mg/kg) and in *Citrus reticulata* (6.27±0.10 mg/kg), respectively. The high value of Zn in L1 might be due to application of manures and fertilizers to the soil. It might also be due to vehicular emissions. The fragmentation of tyres has been implicated for higher concentrations of Zn in heavy traffic zones [25]. Exhaust emissions have been identified as primary source of metallic nuisance [35] such as zinc. Trees in cities are more prone to HM pollution due to pervasive pressure of auto vehicular emissions [36] and plant leaf is the most sensitive part to be affected by air pollutants as major physiological processes are concentrated in the leaf [37]. This explains the reason for the bio-magnification of metals in the plant samples of this study. Zinc plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenase, proteinases, peptidases and phospohydrolases [38]. It (zinc) balances copper (Cu) in the body and is essential for male reproductive activity [21]. Zinc deficiency causes anaemia and retardation of growth and development [39] in human. The consumption of *Citrus reticulata* by anaemic patient in Uyo metropolis might reduce the adverse effect of anaemia due to high content of Zn in these plants (Table 2). Notwithstanding this, dietary intakes of *Citrus* fruits have to be maintained at regulatory limits, as excesses may result in poisoning or toxicity. The concentration of Zn in the soil and fruits was found in the range of 4.41±0.34 to 6.27±0.10mg/kg. The level of Zn in the soil of this study is lower than 9.0 - 400 mg/kg obtained in urban soils of Poznan municipality, Poland [40], 0.90 - 169 mg/kg in Florida soils [41], 58 - 330 mg/kg in roadside soils of Dortmund, Germany (Munch, 1992) but higher than 0.774±0.070 mg/kg in soils of Malaysia [38]. The range of Zn in the fruits is lower than 38.8±0.05 - 60.0±0.17 of Zn in aerial parts of *Polygonatum verticillatum* [42] and 9.02±1.40 to 34.58±2.07 mg/kg, [43] but higher than 0.02±0.10 - 0.06±0.10 mg/g in *Saba florida* in Nigeria [25].

The highest concentrations of Cu in soil and fruit samples were found in L1 (1.896±0.128mg/kg) and *Citrus sinensis* (1.14±0.08mg/kg) respectively. Copper (Cu) like zinc, copper is a component of many enzymes in the plant and plays a role in energy metabolism [44]. The highest concentration of Mn in plants was obtained in *Citrus reticulata* (17.31±2.38mg/kg). The high value of Mn in this study might be due to the application of manure. This is in agreement with the findings of [45]. The pattern of result of heavy metals in *Citrus* fruits shows that Mn> Zn>Pb> Cd > Cu.

4. Relationship Between Heavy Metal Contents in Soil and Fruit of Two *Citrus species*

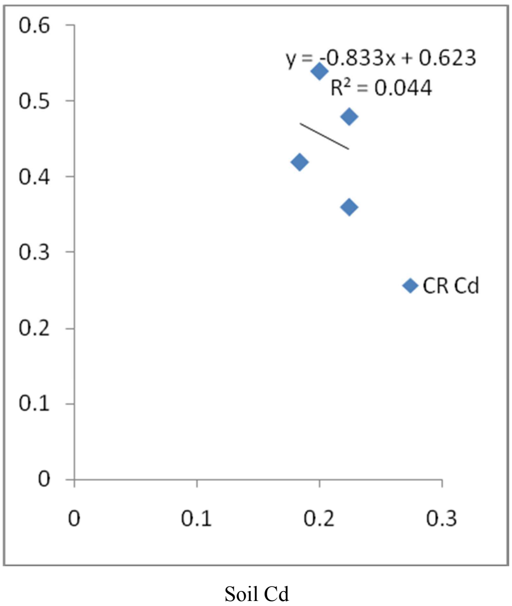

Figure 1. *Relationship between Cd content in soil and Citrus reticulata (CR).*

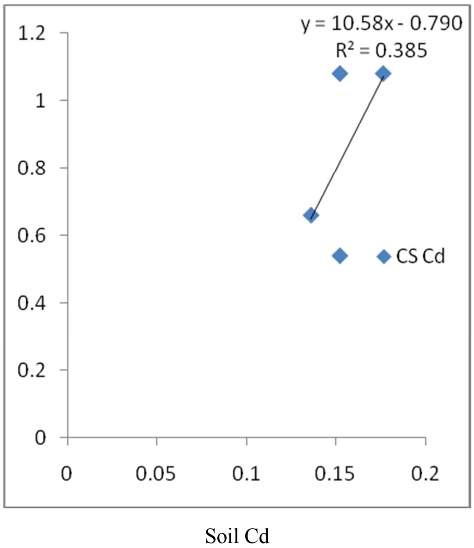

Figure 2. *Relationship between Cd content in soil and Citrus sinensis (CS).*

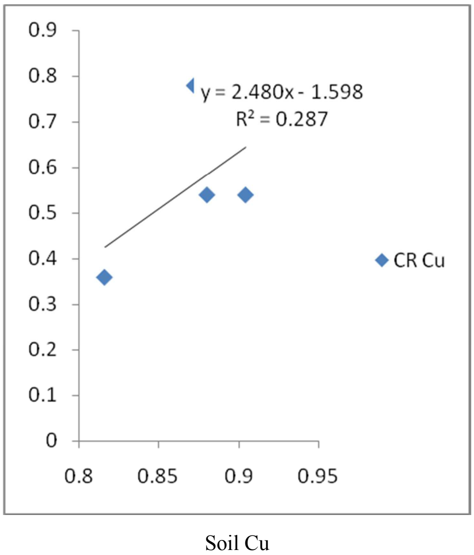

Figure 3. *Relationship between Cu content in soil and Citrus reticulata (CR).*

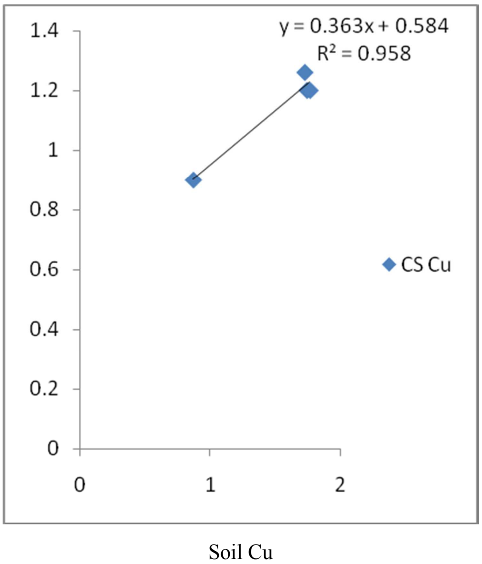

Figure 4. *Relationship between Cu content in soil and Citrus sinensis (CS).*

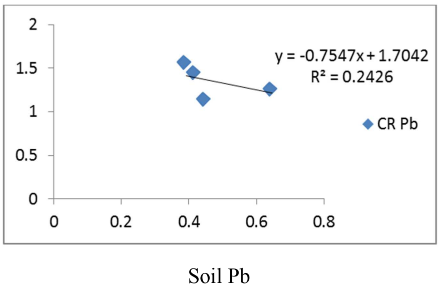

Figure 5. *Relationship between Pb content in soil and Citrus reticulata (CR).*

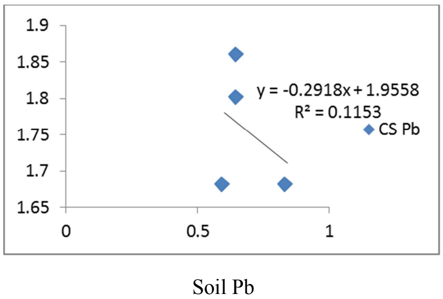

Figure 6. *Relationship between Pb content in soil and Citrus sinensis (CS).*

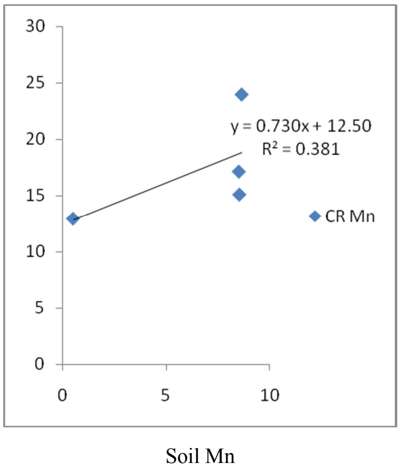

Figure 7. *Relationship between Mn content in soil and Citrus reticulata (CR).*

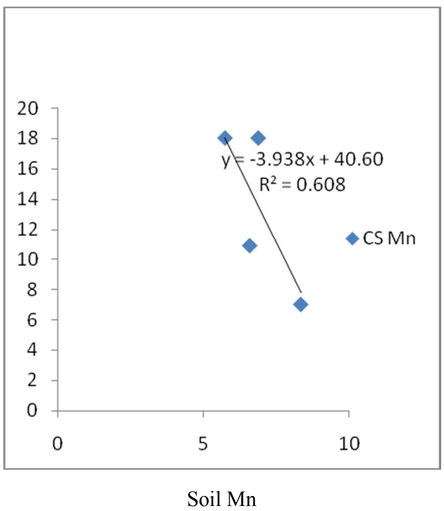

Figure 8. *Relationship between Mn content in soil and Citrus sinensis (CS).*

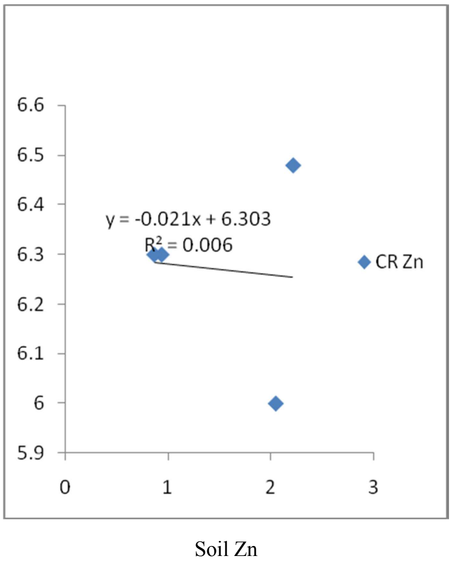

Figure 9. *Relationship between Zn content in soil and Citrus reticulata (CR).*

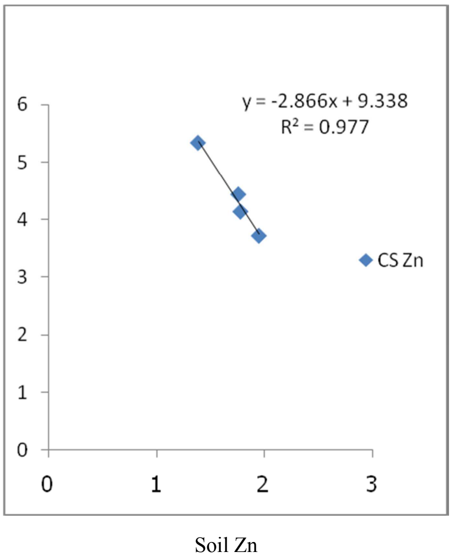

Figure 10. *Relationship between Zn content in soil and Citrus sinensis (CS).*

Regression graphs: Figures 1-10 are regression graphs showing the relationships between soil and fruit heavy metal (Cd, Cu, Pb, Mn and Zn) contents of *Citrus reticulata* and *Citrus sinensis*. It shows the positive slopes observed in soil and fruit relationship graph of Cu and Mn of *Citrus reticulata;* Cd and Cu of *Citrus sinensis* indicate that these plants have the ability to store these metals, which implies that the increase in soil heavy metal contents will result in more bioaccumulation in fruits. The positive slopes also imply that the source of these metals is from the study soils. The negative slopes signify that the plants have no ability for bioaccumulation and also the elevated level of these metals in fruit might have resulted from different sources of pollution other than the soil. [46] also attributed positive relationships between soil and plant heavy metal contents to the study areas and negative relationships to other sources of pollution. From the results, *Citrus reticulata* and *Citrus sinensis* have the inherent ability to take up metals.

5. Conclusion

The metals (Cd, Cu, Mn, Pb and Zn) investigated in this study were detected in all the soils and were within the recommended safe limits of heavy metals by World Health Organization (W.H.O.) Standards. This research also shows that heavy metal concentrations varied among the tested fruits, which reflect the differences in their uptake capabilities. The mean concentrations of Cd were low in the soil but were biomagnified in the plant samples. Conclusively, there were complex relationships among the heavy metal contents in the soil and fruits of this study, therefore it is recommended that *Citrus* farms should not be sited too close to highways and recommended dosage of agrochemicals must be used. The concentration of the heavy metals in the *Citrus* fruit and soil samples in this study could serve as a baseline data for the assessment of agricultural and vehicular exhaust pollutants in soils and *Citrus* fruit obtained in Akwa Ibom State of Nigeria. There should be regular monitoring of toxic heavy metal levels in soil, sewage and foods, to prevent their excessive build-up in the food chain. Further research is required to determine the heavy metal contents of soil and fruit grown in other *Citrus*- growing regions of the country for sustainable citrus production. The information obtained from this research could be essential for a better *Citrus* production, town planning, management and conservation of our vegetation ecosystems.

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